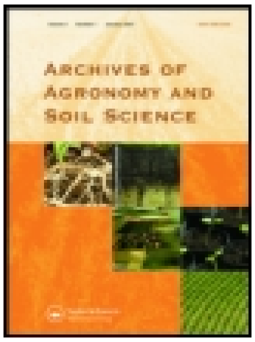

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ARTICLE

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Spring camelina N rate: balancing agronomics and environmental risk in United States Corn Belt

Jane M. F. Johnson, Russ W. Gesch and Nancy W. Barbour

USDA-Agricultural Research Service-North Central Soil Conservation Research Laboratory, Morris, MN, USA

ABSTRACT

Camelina (*Camelina sativa* (L.) Crantz) seed oil has desirable properties for producing advanced biofuels and as a healthy cooking oil. It has been grown for centuries, but basic recommendations for nitrogen (N) fertilizer requirements are still needed to support widespread industrial cultivation across North America. A replicated N-response plot-scale study was conducted on a northern Mollisol soil for two growing seasons to 1) determine seed and oil yield, seed oil content, and vegetative response; 2) determine indices of N use efficiency; and 3) measure post-harvest residual inorganic soil N as an index of environmental risk. Seed and oil yield response to N fertilization was described with a quadratic function, which predicted maximum seed yield (1450 kg ha^{-1}) and oil yield (580 kg ha^{-1}) at about 130 kg N ha^{-1}. However, seed and oil yield did not differ significantly among N-rates above 34 kg N ha^{-1}. Seed oil content averaged 400 g kg^{-1} among all N rates. Agronomic efficiency declined above 34 kg N ha^{-1}, which coincided with an increase of post-harvest soil nitrate-N plus ammonium-N (residual N). Considering N use efficiency, simple cost analysis, and risk associated with residual N, a rate of 34 kg N ha^{-1} is recommended.

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Agronomic efficiency; soil nitrogen; camelina; nitrogen use efficiency; alternative crops

Introduction

Spring camelina (*Camelina sativa* (L.) Crantz) seed oil is suitable for multiple food and industrial uses (Budin et al. 1995; Zubr 1997; Ní Eidhin et al. 2003).[1] Camelina seed oil has been identified as a potential biodiesel feedstock (Bernardo et al. 2003; Fröhlich and Rice 2005; Paulsen et al. 2011), and as a suitable aviation fuel source (Li and Mupondwa 2014).[2] Economic viability and a market chain, as well as sound agronomic guidelines, are needed to support farmer adoption of camelina (Keske et al. 2013).

Several studies (Pavlista et al. 2011; Schillinger et al. 2012; Aiken et al. 2015; Chen et al. 2015; Gesch et al. 2017) have provided various agronomic (e.g., planting methods and timing) recommendations at several locations across Canada and the USA, and N application guidelines are emerging. For example, N fertilizer needs have been assessed in Chile (Solis et al. 2013) and at several North American locations with varying climatic conditions associated with eastern and western Canada (Jiang et al. 2013; Malhi et al. 2014), Northern Great Plains USA (Sintim et al. 2015; Keshavarz-Afshar et al. 2016; Mohammed et al. 2017), and the Pacific Northwest USA (Roseberg and Shuck 2009; Wysocki et al. 2013). However, N fertilizer assessment from the northern Corn Belt are needed.

In general, field-grown camelina seed and oil yield respond positively to N fertilization, although seed oil content may decrease (Solis et al. 2013; Malhi et al. 2014; Sintim et al. 2015; Keshavarz-Afshar et al. 2016; Yiang and Caldwell 2016). Keshavarz-Afshar et al. (2016) demonstrated that although camelina seed oil content declined with applied N but because seed yield increased, seed oil yield

CONTACT Jane M. F. Johnson Jane.Johnson@ars.usda.gov USDA-Agricultural Research Service-North Central Soil Conservation Research Laboratory, Morris, MN, U.S.A.

remained constant. A study from Oregon found neither seed nor oil yield responded significantly to N rates ranging from 0 to 135 kg N ha^{-1} (Roseberg and Shuck 2009), although in this study the data were not analyzed by regression. Regressing the mean oil or seed yield data from their study suggests that it could have fit a linear function to describe a N response. A study from the Pacific Northwest USA across 12 site-years showed that camelina responded linearly to N fertility between 0 and 90 kg N ha^{-1}, allowing for a prediction of 100 kg seed yield for every 12 kg N ha^{-1} applied (Wysocki et al. 2013). Other studies have reported non-linear responses to N rates. For example, in Montana, optimum seed (1190 kg ha^{-1}) and oil (433 kg ha^{-1}) yield corresponded to 45 kg N ha^{-1} with little or no gain at higher N rates (Mohammed et al. 2017). Camelina grown in Wyoming did not appreciably increase in yield with more than 56 kg N ha^{-1} in either year of a two-year study with maximum seed yields of 1000 kg ha^{-1} and 1600 kg ha^{-1} in 2013 and 2014, respectively (Sintim et al. 2015). Regression analysis across four environments in South Central Chile demonstrated maximum predicted yield occurred with 185 kg N ha^{-1}, with maximum yields ranging from 1840 to 2390 kg ha^{-1} (Solis et al. 2013). In Eastern Canada, maximum seed yield (about 1700 kg ha^{-1}) corresponded with 200 kg N ha^{-1} (Yiang and Caldwell 2016), and in western Canada maximum seed yield (2013 kg ha^{-1}) was measured at 170 kg N ha^{-1} (Malhi et al. 2014). As noted by Mohammed et al. (2017), site specific recommendations are important.

Gesch (2014) reported spring camelina yields as high as 2300 kg ha^{-1} in the northern Corn Belt USA, but N fertilizer guidelines for this region are lacking. Research on N fertility for spring camelina has focused on maximizing seed and oil yield response, but most studies lack information on balancing agronomic performance against environmental risk associated with nitrate-N and ammonium-N remaining in the soil after harvest, which can be transported off-site (Randall et al. 1997). Nitrogen application should provide enough N to optimize yield while minimizing any unused N that could be susceptible to leaching or gaseous loss (e.g., nitrous oxide) (Robertson and Vitousek 2009). A study done in Montana, an areas with a semi-arid climate, reported that surface applied super-urea did not appreciably reduce ammonia volatilization compared to urea, but did improve the movement of N into the soil (Keshavarz-Afshar et al. 2017). The northern Corn Belt region USA is characterized by a moist continental temperate climate with (Kottek et al. 2006) Mollisol soils rich in organic matter (USDA-NRCS 2007). It is important to consider the risk of off-site movement of nitrate-N when establishing N fertility guidelines in moist climates compared to semi-arid to arid environments (Benson et al. 1992).

Development of environmentally sound and agronomically profitable fertilizer recommendations is aided by using indices of N use efficiency (Wysocki et al. 2013). Because such indices provide insight into plant N uptake and its conversion into desired agronomic product (Moll et al. 1982; Ciampitti and Vyn 2011; Woli et al. 2016). Information is needed to specifically address camelina's response to N in concert with estimations of N use efficiency and determination of residual inorganic soil N (nitrate-N and ammonium-N). Therefore, the objectives of this study were to 1) determine camelina seed and oil yield, seed oil content, and plant growth response to N applied as urea; 2) estimate indices of N use efficiency of seed and oil production; and 3) measure post-harvest residual soil N as nitrate-N and ammonium-N from field-grown camelina. The goal is to provide N fertilizer recommendations for spring camelina that are relevant to areas with similar soils and continental climates, which support agronomic performance while minimizing loss of reactive forms of N to the environment.

Material and methods

Study sites and experimental design

During two growing seasons (2014 and 2015) replicated plot studies were conducted at the Swan Lake Research Farm in West Central Minnesota (elevation 344 m, 45°35′ N, 95°54′W), which is within the northern Corn Belt region of the USA. New sites were established each year on Barnes clay loam soil (Fine-loamy, mixed, superactive, frigid Calcic Hapludoll), which has about 25 g kg^{-1} soil organic C in the surface 30 cm (Johnson et al. 2016). A randomized complete block design was used with five N levels (treatments) and four blocks (replications) for 20 (3 m by 4.6 m) plots each with 15 rows. The study area including inter-plot border areas was 929 m^2. A previous greenhouse study with camelina suggested 200 kg N ha^{-1} may be needed to observe a curvilinear response (Johnson and Gesch 2013), therefore the rates chosen were 0, 34, 67, 134 and 202 kg N ha^{-1}.

Fertilizer treatments and cultural practices

During the growing season prior to the N rate study, sorghum/Sudan grass (*Sorghum bicolor* × *Sorghum bicolor* var. sudanense) was planted at 50.2 plants m^{-2} in 38 cm rows without N fertilizer, then harvested twice to reduce plant available forms of soil N (nitrate-N and ammonium-N). Preplant (0–60 cm) soil nitrate-N plus ammonium-N averaged 28 kg N ha^{-1} in 2014 and 31 kg N ha^{-1} in 2015. Soil tests indicated soil K was at 145 mg kg^{-1}, since this is considered high for Minnesota (Kaiser et al. 2016) no K was applied. The day before planting urea was applied with a hand spreader to achieve the desired N-rates, plus all plots received 33.6 kg ha^{-1} of super (triple) phosphate (P_2O_5) 0–44-0 as some plots had low to very low soil test P values (Kaiser and Lamb 2012), and the plots received a pre-emergence trifluralin (a,a,a-Trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine) at 1.1 kg a.i. ha^{-1} for weed control. To incorporate the ag-chemicals and prepare the seedbed, all plots were tilled with a cultivator (15 cm depth) and two passes with a drag. Camelina was planted 28 May 2014 and 21 May 2015 at a seeding rate of 3.5 kg ha^{-1} in 19 cm spaced rows using a no-till drill (model 3P1006NT, Great Plains, Salina, KS).

Yield

Total aboveground plant dry mass, seed yield, and harvest index were calculated using hand-thrashed samples collected within a 1-m^2 quadrant one day prior to mechanically harvesting plants for seed yield. When silicles had turned brown and were dry (20 August 2014 and 11 August 2015) plants were harvested on from the center eight-rows in each plot using a plot combine. Dry seed yield (kg ha^{-1}) was determined after drying samples to a constant weight at 65°C in a forced air oven and screen cleaning. Seed oil content (g kg^{-1}) was measured by pulsed nuclear magnetic resonance (Bruker Minispec mq10, Bruker, The Woodlands, TX). The instrument was calibrated with known quantities of pure camelina oil as described for other oil seeds crops (Gesch et al. 2005). According to the AOCS Method 2–75, samples (approximately 5-g each) were dried at 130°C for 4 h and cooled in a desiccator for 15 min before measuring oil content. Oil yield (kg ha^{-1}) was calculated by multiplying seed oil content fraction (wt wt^{-1}) by dry seed yield.

Plant and soil samples measurement and analysis

Stand counts were determined by counting the number of plants in 1-m row length. In 2014 plant stands were counted four times (11, 18, 23, and 30 June), while in 2015 they were counted twice (10 and 16 June). Reported populations are based on the final stand counts.

Root to shoot ratio, root biomass, and corresponding shoot biomass were determined on samples collected on 10 July 2014 and 30 July 2015 when plants were transitioning from vegetative to reproductive growth. Shoots were collected within two 1-m row lengths for a total of 0.381 m^2 area. Roots were collected using a 6.5 cm diameter tipped hydraulic probe to a depth of 60 cm. Previous work found that the vast majority of the roots occurred within this rooting depth (Gesch and Johnson 2015). Briefly, two root cores were taken from each area where shoot biomass was sampled for a total of four cores per plot. One core in each area was over the row and one core was between the rows to capture anticipated nonuniform root architecture. Soil cores were stored at 4°C in plastic bags until roots could be hand-washed as previously described by Johnson and Gesch (2013) using a modification of the method developed Smucker et al. (1982). Root biomass (kg ha^{-1}) was expressed as the total from all cores within a plot for the 0–60 cm profile. Plant samples were dried at 65°C in a forced air oven to a constant mass and ground to pass through 0.425-mm screen. Carbon and N content were determined on a 200-mg sample by combustion using a LECO TRU SPEC CN Analyzer (LECO Corporation, St. Joseph, MI). Only N data is presented.

Soil samples (one 4-cm i.d., probe per plot) were taken in the spring (4 June 2014 and 27 May 2015) incrementally (0–15, 15–30, and 30–60 cm) and confirmed that the desired range of N had been achieved (data not shown). Post-harvest soil samples at the same depth intervals were taken on 22 August 2014 and 12 August 2015 to measure of residual soil N (nitrate-N and ammonium-N). The N in these post-harvest samples represents the reactive-N at risk of being lost to the environment during the non-growing season.

Soil samples were dried to a constant mass at 37°C for chemical analysis or at 105°C for bulk density determination. Visible roots and plant residue were removed from the samples for chemical analysis, then samples were ground in a hammer mill before finally pulverizing with a ball mill (Frisch Pulvesette 5 Idar-Oberstein, Germany). Soil analyses included nitrate-N and ammonium-N content using the method of Mulvaney (1996), Olsen P using the method by Kuo (1996), and extractable K using the method of Helmke and Sparks (1996).

Calculations and statistical analysis

Harvest index was calculated as dry seed biomass per total shoot biomass at harvest. Four indices of N use efficiency are reported. Seed N use was calculated with Equation. (1) based on Moll et al. (1982), which is a measure of the efficiency to convert N to seed biomass. Seed N and oil N use efficiency were calculated with Equation. (2) and Equation. (3), respectively, which are comparable to N use efficiency calculated by Ciampitti and Vyn (2011) and Woli et al. (2016) called partial factor productivity. Agronomic efficiency calculated with Equation. (4) is the increase in seed yield with N compared to control without N applied per unit N applied (Woli et al. 2016).

$$\text{Seed N use } (\text{kg kg}^{-1}) = \text{Dry seed yield}/\text{Seed N uptake} \tag{1}$$

$$\text{Seed N use efficiency } (\text{kg kg}^{-1}) = \text{Dry seed yield}/\text{Seed N applied} \tag{2}$$

$$\text{Oil N use efficiency } (\text{kg kg}^{-1}) = \text{Oil yield }/\text{N applied} \tag{3}$$

$$\text{Agronomic efficiency } (\Delta \text{ kg kg}^{-1}) = (\text{Dry seed yield}_{\text{N}} - \text{Dry seed yield}_{\text{No N}})/\text{N applied} \tag{4}$$

Measured parameters were analysed for linear and quadratic response to the continuous variable N fertilizer rate based on individual data points from both years and by year using PROC REG in SAS/STAT software version 9.4 (SAS Institute 2012). Regression analysis allowed us to determine the inflection point for the maximum fertilizer response. Regressions presented are the individual data from both years with exceptions explicitly noted when regressions are for individual years. In addition, Proc GLM was used to test differences among N fertilizer rates, years, and N-rate by year interaction. Mean separations were made using the probability of differences among least squares means. Comparisons are considered statistically significant at a P value of ≤ 0.05.

Results

Growing season conditions

In 2014 and 2015, May to September (growing season) precipitation was 360 mm and 378 mm, and average daily mean temperatures were 18.2 and 19.0°C, respectively (Table 1). In 2014, May and June received 64 mm more precipitation while July through September received 135 mm less rain

Table 1. May through September monthly mean precipitation, and monthly mean daily temperatures for 2014, 2015, and long-term normal (1981 to 2010).

	Precipitation			Daily mean temperature		
	2014	*2015*	*normal* [a]	*2014*	*2015*	*normal*
Month	– – – – – – - mm – – – – – –			– – – – – – – °C – – – – – – -		
May	89	149	72	14.1	13.9	13.8
June	149	38	102	20.0	20.3	19.0
July	33	74	99	20.1	21.8	21.3
August	73	85	85	21.0	20.1	20.1
September	17	32	74	15.6	19.0	15.0
Total mm/average °C	360	378	431	18.2	19.0	17.8

[a]30-year (1981–2010) normal measured at West Central Research and Outreach Center (Arguez et al. 2010) located approximately 24 km from the study site.

compared to the 30 year (1981–2010) normal for the region (Arguez et al. 2010). In 2015, May had 77 mm more precipitation while June, July, and September had 131 mm less, and August had similar precipitation compared to the 30-year normal. The growing seasons of 2014 and 2015 were both warmer than the 30-year normal.

Seed and oil yield

Seed yield and seed oil yield responded significantly to N application (Table 2, Figure 1A and 1B). Maximum seed and oil yields were similar both years, averaging 1400 kg ha^{-1} and 1500 kg ha^{-1} and 560 kg ha^{-1} and 610 kg ha^{-1} in 2014 and 2015, respectively. Yield response could be described by either a linear or quadratic function, but a quadratic function resulted in a greater r^2 (Table 2, Figure 1A and Figure 1B). Using the coefficients from the quadratic function based on both years' data, the calculated maximum seed yield (1450 kg ha^{-1}) was predicted at 130 kg N ha^{-1} and maximum oil yield (580 kg ha^{-1}) at 126 kg N ha^{-1}. Averaging yield data from both years, neither seed nor oil yield increased significantly ($P \le 0.05$) above the 34-kg N ha^{-1} application rate (Figure 1A and 1B).

Seed oil content averaged 400 g kg^{-1} among treatments and years (Figure 1C). A N-rate by year interaction was found for seed oil content (Table 2, Figure 1C). In 2014, seed oil content showed no response to addition of N fertilizer, as neither a linear nor quadratic function was significant. In 2015, seed oil content declined with N application and could be described with either a linear or quadratic function. Comparison of 2015 mean values indicated that seed oil content at the 0 and 34 kg N ha^{-1} application rates were similar, and both were greater than the other three N rate treatments.

Table 2. Camelina seed yield (0% Moisture), seed oil yield and seed oil content, probability of N rate, and significance of linear and quadratic models (n = 40), Figure 1 for significant quadratic equations. Regression for individual years shown only when interaction significant (n = 20). Levels of statistical significance used are: * P < 0.05, ** P < 0.01, ***P < 0.001, and **** P < 0.0001, ns = not significant.

	Seed yield (0% Moisture)		Seed oil yield		Seed oil content			
Source	– – – – – – – - kg yield ha^{-1} – – – – –				– – g kg^{-1} ––			
Rate	**		**		ns			
Year	ns		ns		ns			
Year × Rate	ns		ns		*			
Model	r^2	P	r^2	P	r^2		P	
Linear	0.146	**	0.121	*	0.035		ns	
	Y = 1210 + 1.115X		Y = 493 + 0.4127X					
Quadratic	0.293	**	0.259	**	0.036		ns	
					2014		2015	
Model					r^2	P	r^2	P
Linear					0.05	ns	0.452	***
							Y = 414–0.8X	
Quadratic					0.105	ns	0.561	***

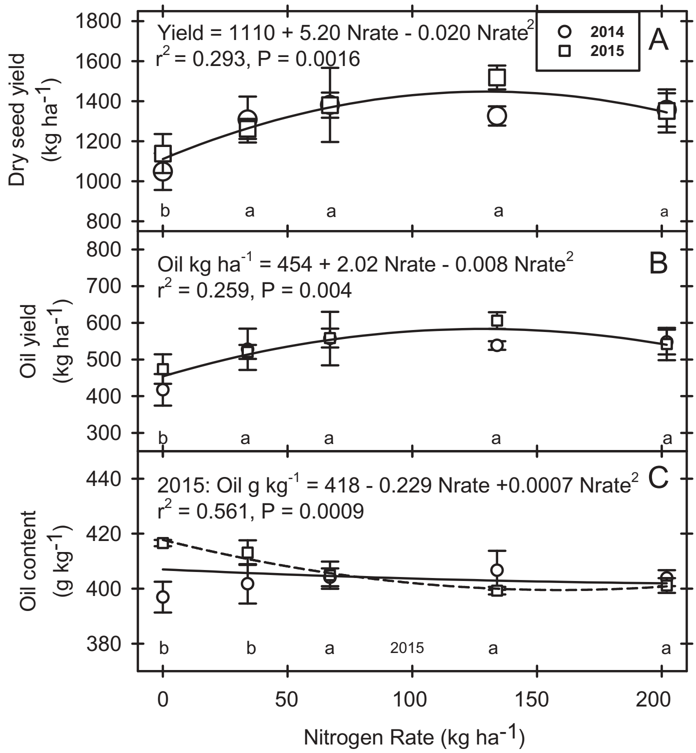

Figure 1. Camelina seed yield (A), seed oil yield (B), and oil content (C) as a function of N fertilizer rate. Seed yield, and oil yield, and oil content quadratic response equations were determined using raw data (n = 40) from both years (Solid line). For oil content, a quadratic equation based on only 2015 data is also shown (Dashed line). Symbols represent treatment means each year (n = 4) with standard error bars. Different letters at the bottom of each graph denote significance among N rates, $P \leq 0.05$. For oil content the letters only apply to 2015, since in 2014 rate was not significant.

Both seed N Equation. (2) and oil N Equation. (3) use efficiencies were described using either a linear or quadratic function (Table 3). The quadratic function of both had a higher r^2 compared to the linear regression. Agronomic efficiency Equation. (4) dropped 25% between 34 and 67 kg N ha^{-1}, 43% between 67 and 134 kg N ha^{-1} and 47% between 134 and 202 kg N ha^{-1} (Table 3). Thus, yield return per unit N applied declined dramatically above 67 kg N ha^{-1}. Although agronomic efficiency was calculated from seed yield, it is also applicable to oil yield because oil content was constant among N rates. Harvest index did not respond to N fertilizer application and was similar both years, averaging 0.35 among all rates and years (data not shown). Seed N use Equation. (1) was similar at all N rates both years, with an overall average of 22 kg seed produced kg^{-1} N uptake (data not shown).

Vegetative growth response

Vegetative shoot growth responded positively to N application and was best described by quadratic function (Table 4). Consistent with a nonlinear response, the greatest shoot biomass (1870 kg ha^{-1}) was measured at 67 kg N ha^{-1}, which was comparable to the 134 kg N ha^{-1} rate but differed from the other rates. Root biomass (0–60 cm) did not respond to N fertilizer application, averaging 1050 kg ha^{-1} across N rates. Root:shoot ratio decreased at all applied N rates, and was described significantly by a quadratic function. The observed response was the result of changes in shoot biomass rather than root biomass. At the 202 kg N ha^{-1} rate, no significant difference was detected even though the shoot biomass declined while root biomass did not. Shoot tissue N content was described with a linear and a quadratic function. It increased at the two highest application rates

Table 3. Camelina seed nitrogen (N) use efficiency, oil N use efficiency, seed agronomic efficiency, probability of N rate, and significance of linear and quadratic models (n = 40). Levels of statistical significance used are: * P <0.05, ** P <0.01, ***P <0.001, and **** P <0.0001. Values in a column followed by different letters differ at P ≤0.05.

N applied	Seed N use efficiency [a]		Oil N use efficiency [b]		Agronomic efficiency [c]
kg N ha^{-1}	– – – – – – – – -kg yield kg^{-1} N applied – – – – – – – – –				– – Δkg kg^{-1} ––
34	38.2 ± 1.75a		15.6 ± 0.820a		5.71
67	20.5 ± 1.35b		8.30 ± 0.534b		4.28
134	10.6 ± 0.378c		4.26 ± 0.130c		2.45
202	6.72 ± 0.313d		2.70 ± 0.127d		1.29
Source					
Rate	****		****		Not applicable[d]
Year	ns		ns		
Year × Rate	ns		ns		
Model	r^2	P	r^2	P	
Linear	0.783	****	0.7730	****	
	Y = -0.17X+ 37.8		Y = −0.07X + 15.4		
Quadratic	0.916	****	0.907	****	
	$Y = 1.6 \times 10^{-3}X^2 - 0.540X + 52.95$		$Y = 6.4 \times 10^{-4}X^2 - 0.222X + 21.66$		

[a]Seed N use efficiency factor (Eq. 2) is seed yield (at 0% per moisture) per kg N applied (Ciampitti and Vyn 2011), which corresponds to partial factor efficiency used byy Woli et al. (2016).
[b]Oil N use efficiency factor (Eq. 3) is oil yield per kg N applied (Ciampitti and Vyn 2011), which corresponds to partial factor efficiency used by Woli et al. (2016).
[c]Agronomic efficiency (Eq. 4) is the increase in seed yield with N compared to control without N applied per unit N (Woli et al. 2016).
[d]Not applicable because this parameter was calculated from treatment means.

but was similar among 0, 34, and 67 kg N ha^{-1}. Root tissue N content did not respond to N rate, averaging 16.3 mg N g^{-1}, which suggests that N is transported to the shoot to be assimilated or stored. Final plant stand varied between years with 71 ± 15 plants m^{-2} and 1,70 ± 28 plants m^{-2} in 2014 and 2015, respectively but did not differ among N application rates (data not shown).

Nitrogen uptake

Nitrogen uptake (kg N ha^{-1}) in the vegetative plant parts and harvested seeds (Table 5) was calculated from the corresponding N concentration of the biomass (Table 4). Vegetative shoot N uptake, total biomass N, and seed N uptake at maturity were described using a linear or quadratic function. The uptake of N by roots did not change because neither root biomass nor root N content responded to N application. The midseason vegetative and harvested seed N uptake are not additive because N in the vegetation at midseason was translocated into the seed. Less vegetative (shoot and root) N uptake occurred in 2014 than in 2015 while the N uptake in harvested seed was similar between years.

Post-harvest soil

The amount of residual soil inorganic N after harvesting camelina responded in a linear manner to N rate (Figure 2). The quadratic functions were significant but provided little or no improvement to describing the response, so only linear responses are shown. Based on treatment means, the two highest N rates contained significantly more residual inorganic soil N than the 0 rate at the 0–15 and 15–30 cm depths. Whereas, the two lowest rates were not different from the control. In the 30–60 cm depth only the 202 kg N ha^{-1} rate had significantly more residual inorganic N than the control. The greatest amount of N was in the surface 0–15 cm. Significantly more residual soil N was measured after harvest in 2014 compared to in 2015 at all depth intervals but the difference was largest within the 0–15 cm depth interval.

Table 4. Midseason camelina vegetative growth response and plant tissue N content. Levels of statistical significance used are: * $P < 0.05$, ** $P < 0.01$, ***$P < 0.001$, and **** $P < 0.0001$. Values in a column followed by different letter differ at $P \leq 0.05$.

N applied	Shoot		Root		Root:shoot		Shoot		Root	
kg N ha^{-1}	– – – – – – kg ha^{-1} – – – – –						– – – – – – – – -mg N g^{-1} – – – – – – – –			
0	1090 ± 243c		1010 ± 123		1.15 ± 0.176a		38.1 ± 2.27b		16.0 ± 0.698	
34	1490 ± 189b		923 ± 120		0.682 ± 0.0991b		36.7 ± 1.80b		16.3 ± 0.310	
67	1870 ± 171a		1120 ± 109		0.622 ± 0.0577b		40.4 ± 1.21b		17.2 ± 0.751	
134	1810 ± 149ab		1150 ± 107		0.647 ± 0.0544b		45.0 ± 1.84a		15.9 ± 0.406	
202	1440 ± 128b		1050 ± 117		0.809 ± 0.134b		45.1 ± 2.03a		16.3 ± 0.613	
Year										
2014	1240 ± 122b		853 ± 40.2b		0.840 ± 0.096		41.5 ± 0.93a		15.8 ± 0.294a	
2015	1840 ± 88.5a		1250 ± 68.3a		0.721 ± 0.061		40.7 ± 1.71a		16.9 ± 0.395a	
Source										
Rate	***		ns		***		***		ns	
Year	****		****		ns		ns		*	
Year× rate	ns		ns		ns		*		ns	
Model	r^2	P	r^2	P	r^2	P	r^2	P	r^2	P
Linear	0.031	ns	0.016	ns	0.050	ns	0.230	***	0.0063	ns
							$Y = 0.038X + 37.5$			
Quadratic	0.254	**	0.033	ns	0.262	**	0.244	***	0.0348	ns
	$Y = -6.54 \times 10^{-2}X^2 + 14.6X + 1104$				$Y = 4.04 \times 10^{-5}X^2 - 9.25x10^{-3}X + 1.072$		$Y = -1.63 \times 10^{-4}X^2 + 0.071X + 36.7$			

Table 5. Midseason camelina vegetative N uptake and N uptake in harvested seed. Levels of statistical significance used are: * $P < 0.05$, ** $P < 0.01$, ***$P < 0.001$, and **** $P < 0.0001$. Values in a column followed by different letter differ at $P \leq 0.05$.

N applied	Shoot N		Root N		Total biomass N [c]		Seed N	
kg N ha^{-1}			‒ ‒ ‒ ‒ ‒ ‒ ‒ ‒ ‒ ‒	‒kg N ha^{-1} ‒ ‒ ‒ ‒	‒ ‒ ‒ ‒ ‒ ‒			
	39.8 ± 8.25c		15.1 ± 1.75a		54.9 ± 9.69c		49.5 ± 2.48c	
34	54.0 ± 6.63b		14.1 ± 1.74a		68.1 ± 6.62cb		58.5 ± 2.81b	
67	74.9 ± 6.20ab		18.2 ± 1.53a		93.1 ± 7.20ab		63.5 ± 4.46ab	
134	82.1 ± 6.68a		17.5 ± 1.387a		99.6 ± 9.61a		66.7 ± 2.17a	
202	64.2 ± 5.77b		16.2 ± 1.29a		80.5 ± 5.74b		63.8 ± 2.98ab	
Year								
2014	50.8 ± 4.75b		13.7 ± 0.712b		64.5 ± 5.11b		62.3 ± 2.32a	
2015	75.2 ± 4.87a		18.8 ± 0.913a		93.9 5 ± 04a		58.5 ± 2.26a	
Source								
Rate	****		ns		****		****	
Year	****		****		****		ns	
Year × Rate	ns		ns		ns		ns	
Model	r^2	P	r^2	P	r^2	P	r^2	P
Linear	0.142	*	0.023	ns	0.136	*	0.207	**
	$Y = 0.13X + 52.0$				$Y = 0.135X + 67.4$		$Y = 0.064X + 54.8$	
Quadratic	0.373	***	0.064	ns	0.358	***	0.349	***
	$Y = -2.87 \times 10^{-3}X^2 + 0.711X + 52.95$				$Y = -3.09 \times 10^{-3}X^2 + 0.765X + 52.1$		$Y = -9.44 \times 10^{-4}X^2 + 0.256X + 50.2$	

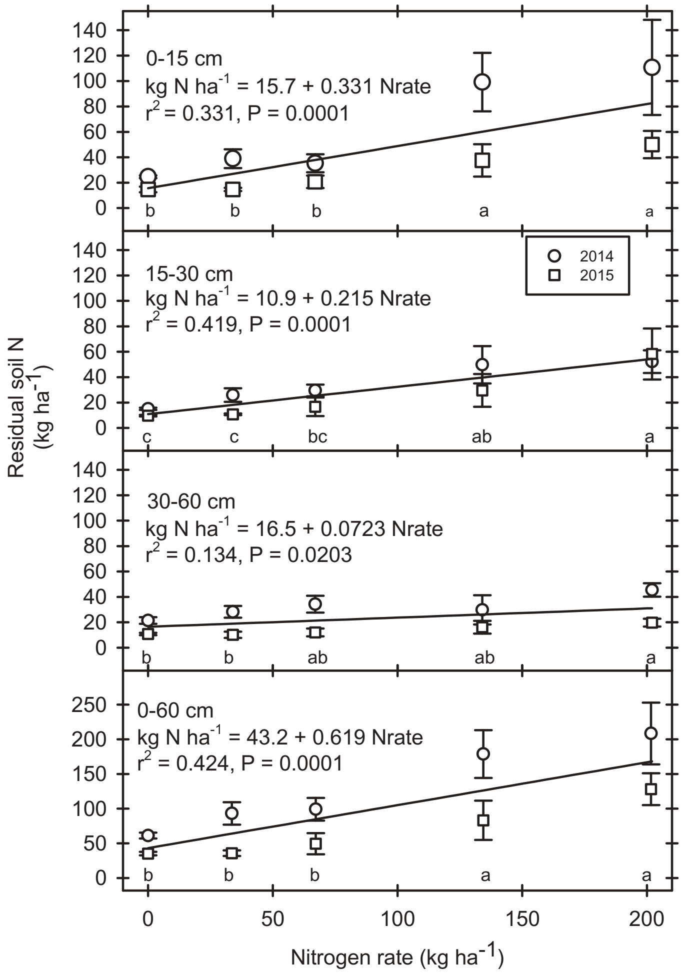

Figure 2. Residual (nitrate-N plus ammonium-N) soil nitrogen (kg N ha^{-1}) at 0–15, 15–30, 30–60 and 0–60 cm profile intervals following camelina harvest in 2014 and 2015. Linear response equations and lines shown were determined using raw data from both years (n = 40). Symbols represent treatment means each year (n = 4). Different letters at the bottom of each graph denote significance among N rates at P ≤ 0.05.

Discussion

The study's goal was to provide a N rate recommendation applicable for optimizing camelina growth and yield while minimizing environmental risks related to residual inorganic N for soils and climate such as those found in the northern Corn Belt USA. The approach taken resulted in a lower N fertilizer (34 kg N ha^{-1}) recommendation than that based on achieving maximum yield (130 kg N ha^{-1}). Camelina seed and oil yield responded positively to N fertilization, similar to a study in the Pacific Northwest (Wysocki et al. 2013; Sintim et al. 2015), which reported a linear response between 0 and 112 kg N ha^{-1}. However, in the current study, camelina responded linearly to applied N up to 134 kg ha^{-1}. A study across four locations in Eastern Canada reported linear and non-linear seed oil yield increase from 0 to 150 kg applied N ha^{-1} depending upon location (Jiang et al. 2014). The highest oil yields in the Eastern Canada study were achieved at 162 to 166 kg applied N ha^{-1}, which is greater than the estimated 130 kg N ha^{-1} for maximum yield calculated in this study. Our maximum average seed yield (1450 kg ha^{-1}) measured was within the range (1000 to 1600 kg ha^{-1}) reported in Wyoming (Sintim et al. 2015), slightly less than observed yields in

Eastern Canada (Yiang and Caldwell 2016), but less than the > 2000 kg ha^{-1} yields reported from Western Canada (Malhi et al. 2014) and South Central Chile (Solis et al. 2013).

Seed oil content did not respond as N rate increased. Declines in camelina seed oil content with increasing N rate have been reported by others (Johnson and Gesch 2013; Solis et al. 2013; Jiang et al. 2014; Sintim et al. 2015; Keshavarz-Afshar et al. 2016). Reductions in seed oil content with increased N have been associated with corresponding increases in protein (Jiang et al. 2014; Keshavarz-Afshar et al. 2016), which is caused by biochemical competition between fatty acid and protein syntheses pathways (Gehringer et al. 2006; Rathke et al. 2006). Similar to the present study, others have reported a lack of response of camelina seed oil content to increasing N application rate in the field (Roseberg and Shuck 2009; Wysocki et al. 2013). Additional research is needed to understand if the divergent oil content responses reported for N fertilization are due to climatic, soil, or genotypic causes.

In the current study, harvest index (0.35) did not respond to N fertilizer. Keshavarz-Afshar et al. (2016) reported that harvest index was 0.40 after fertilizing with stabilized urea, as compared to 0.36 when fertilized with typical urea. Sintim et al. (2015) reported that harvest index responded in a positive quadratic manner to N fertilizer, but the maximum did not exceed 0.30.

Neither root biomass nor root N content responded to applied N, consistent with previous results reported from a greenhouse study (Johnson and Gesch 2013). Pan (2011) reported root dry matter increased with N application between 0 and 50 kg ha^{-1} but no additional increase was observed at higher N rates. In contrast to container-study results (Pan et al. 2011; Johnson and Gesch 2013), field-grown camelina in the present study had a greater root:shoot ratio in the absence of N application compared to when it was applied. A decline in root:shoot ratio with increasing N availability is often caused by greater shoot growth rather than by a change in root growth (Kamh et al. 2005). At midseason, N uptake in the total biomass (roots plus shoot) was approximately 55 kg N ha^{-1} and 68 kg N ha^{-1} in the 0 and the 34 kg N ha^{-1} treatments, respectively (Table 5) demonstrating that mineralization of organic matter can provide N to support camelina growth and yield since the amount of N accounted for in the plant biomass exceeded the N applied. Differences in N fertilizer response across locations may be attributed to differences among soil capacity to provide plant available soil N (nitrate-N and ammonium-N). Wysocki et al. (2013) noted that at locations where the plant available soil N was low, more applied N was required to supply 120 to 130 kg N ha^{-1} (plant-available soil N plus applied N) to optimize agronomic yield on a silt loam soil. We calculated that maximum agronomic yield occurred at 156 kg N ha^{-1} (including nitrate-N and ammonium-N at planting plus applied fertilizer N).

The difference between nitrate-N plus ammonium N remained soil in 2014 (61 kg N ha^{-1}) and in 2015 (35 kg N ha^{-1}) may be attributed to difference in plant uptake and movement of N in the soil. Less nitrogen was taken up by plants in 2014 compared to the 2015, implying more N left behind in the soil. Also, July and August 2014 were drier than the same timeframe in 2015, presumably reducing the opportunity for nitrate-N to move with water deeper into the soil profile at the time sampling. However because more residual N especially water-soluble nitrate remained after harvest this N was still susceptible to being leached into ground water, and/or being transformed into gaseous forms (i.e., nitrous oxide) becoming an environmental liability (Robertson and Vitousek 2009; Zebarth et al. 2009).

In the present study, seed and oil yield N use efficiency declined with increasing N, and agronomic efficiency declined dramatically beyond an application rate of 67 kg N ha^{-1}. Therefore, using only agronomic parameters to determine N rate does not address environmental issues or economic return. Complete economic analyses are beyond the scope of this study. However, using a very simplistic economic analysis assuming a price of \$313.31 Mg^{-1} for urea (http://www.farmersco-op.coop/pages/custom.php?id=21023), 130 kg ha^{-1} N rate for a predicted maximum 1450 kg seed ha^{-1} and maximum 580 kg oil ha^{-1} would cost \$0.028 kg^{-1} of seed or \$0.07 kg^{-1} of oil, respectively. At 34 kg N ha^{-1} and the two-year average yield of 1280 kg seed ha^{-1} and 520 kg oil ha^{-1}, the cost decreases to \$0.008 kg^{-1} of seed and

\$0.020 kg^{-1} of oil, respectively, which is a 3.5-fold cost difference per unit yield. Assuming all other costs remain constant, profit realized from additional N beyond 34 kg N ha^{-1} would decline, suggesting the economically optimum N rate would be less than the agronomic maximum. In the Great Plains, the cost of N fertilizer became the greatest input cost when N rate exceeded 75 kg ha^{-1} (Chen et al. 2015). Comparing N fertilizer rates for corn (*Zea mays* L.) to achieve maximum agronomic yield to those designed to maximize return per unit N applied, demonstrated that over application of N caused a loss on return per unit of N applied (Sawyer et al. 2016; Iowa State University 2018).

Conclusions

This study provides insights into spring camelina N fertilizer requirements for a temperate northern climate. Results indicate that a rate of 34 kg ha^{-1} of applied N was sufficient to achieve economically viable camelina seed and oil yields without leaving too much residual N behind in the soil, making it a reasonable recommendation for the study region. This is justified by the fact that yields did not significantly increase beyond the 34 kg N ha^{-1} rate and that the three efficiency indices were greatest and cost lowest at this level. Nitrogen rates greater than 67 kg ha^{-1} are not recommended because of the reduction in agronomic efficiency and the increased environmental risk associated with nitrate-N and ammonium-N remaining in the soil after harvest. Additional research is needed to identify camelina cultivars with improved N use efficiency and to address timing, placement, and formulation (e.g., controlled-release) of N fertilizers and credits for soil N, which may improve nutrient uptake and conversion efficiency, increase economic return, and minimize undesirable environmental consequences of N overuse. These results are applicable to regions with high organic matter Mollisol soils and continental climates like those found in the northern Corn Belt of the USA.

Notes

1. The USDA is an equal opportunity provider and employer.
2. The use of trade, firm, or corporation names in this publication is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by the United States Department of Agriculture or the Agricultural Research Service of any product or service to the exclusion of others that may be suitable.

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ORCID

Jane M. F. Johnson http://orcid.org/0000-0002-1687-4007

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